PROTOPLASMIC VISCOSITY CHANGES IN DIFFERENT REGIONS OF THE GRASSHOPPER NEUROBLAST DURING MITOSIS

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Protoplasmic viscosity has been studied by a variety of methods. Each has certain advantages and certain limitations, with regard to the kind of living material to which it is best suited and the accuracy of the results that it will give. These methods and their uses have been reviewed critically by Heilbrunn (1928, 1943) and therefore will not be considered in detail here. Of the several that have been employed the brownian movement method is probably best suited to viscosity studies involving limited regions of the single cell. It may be applied in either of two ways: 1) to calculate the absolute viscosity by measuring the displacement in one direction of the molecularly-bombarded particle and the time required to bring this about, certain other characteristics of the cell and its environment being known, or 2) to compare the viscosities of different regions of a cell at different mitotic stages or under different experimental conditions through observations of the relative speeds of brownian movement. The latter has been used in the present investigation.

The neuroblasts of the grasshopper embryo possess several advantages in such a study. Since they are situated on the ventral side of the embryo, they can be brought next to the cover glass in hanging-drop preparations and microscopic observations of the mitotically active, living cells can extend over several hours. All the cell features that are visible in the usual fixed and stained preparation can be seen, and many phases of the mitotic cycle can be identified readily. The cell is relatively large, measuring about 30 μ in diameter. It maintains a visible polarity from one mitotic division to the next, so that a given region can be located in any cell at any stage of division. The cytosome contains large numbers of mitochrondria, which not infrequently find their way into the spindle during mitosis. By observing the brownian movement of these tiny bodies it is possible to compare the viscosities of the surrounding protoplasm of all non-nuclear parts of the cell at all stages of the mitotic cycle.

Material and Methods

Embryos of *Chortophaga viridifasciata* (De Geer) were prepared by the hanging-drop method previously described for this material (Carlson and Hollaender,

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1944). The culture medium, which must be isotonic with the embryonic cells, is made up as follows:

NaCl	0.70	gm.
KCl	0.02	gm.
$CaCl_2$	0.02	gm.
$MgCl_2$	0.01	gm.
NaH_2PO_4	0.02	gm.
$NaHCO_3$	0.005	gm.
Glucose	0.80	gm.
H_2O (pyrex redistilled)	100.0	cc.

The pH of this solution is approximately 6.5, which is about the pH of the grasshopper egg yolk. A small amount of yolk is added to each hanging-drop to provide nitrogenous food materials for the cells.

Preparations were studied in a constant temperature box enclosing all of the microscope except the upper part of the body tube and arm. All observations were made at $26 \pm 0.5°$ C. The light used as a source of microscope illumination was passed through copper sulfate solution to filter out the heat.

Observations were made exclusively on neuroblasts. These cells are shown in representative mitotic stages in Figure 1, which is based on camera lucida sketches of living cells. It will be noted that the neuroblast deviates from the typical cell in two main respects. First, the nucleus is not spherical but roughly hemispherical with a central cytoplasmic core connecting the polar and apolar regions of the cell (Figs. 1*A* and 1*B*). Second, the cell divides unequally to form a small daughter ganglion cell and a large daughter neuroblast (Fig. 1*J*.) The great advantage of these cells is their large size, which makes it possible to observe and study in the living, unstained cell many structural features that are very difficult or even impossible to deal with in smaller cells.

The mitochondria of the neuroblast are spheroidal in shape and measure approximately 0.3–0.5 μ in diameter.

Terminology

Relation of viscosity to brownian movement.

The relation between the rate of brownian movement and viscosity is given by Einstein's equation

$$D_x^2 = \frac{RTt}{N3\pi\eta a}$$

in which D_x is the displacement of the particle along the x axis; R, the gas constant; T, the absolute temperature; t, the time; N, the Avogadro number; a, the radius of the particle; and η the viscosity expressed in poises. Accordingly, the viscosity of the dispersion medium varies inversely as the square of the displacement of a given particle along one axis.

The rapidity of brownian movement of the mitochondria in selected regions of the cell was observed and classified according to the following system. The

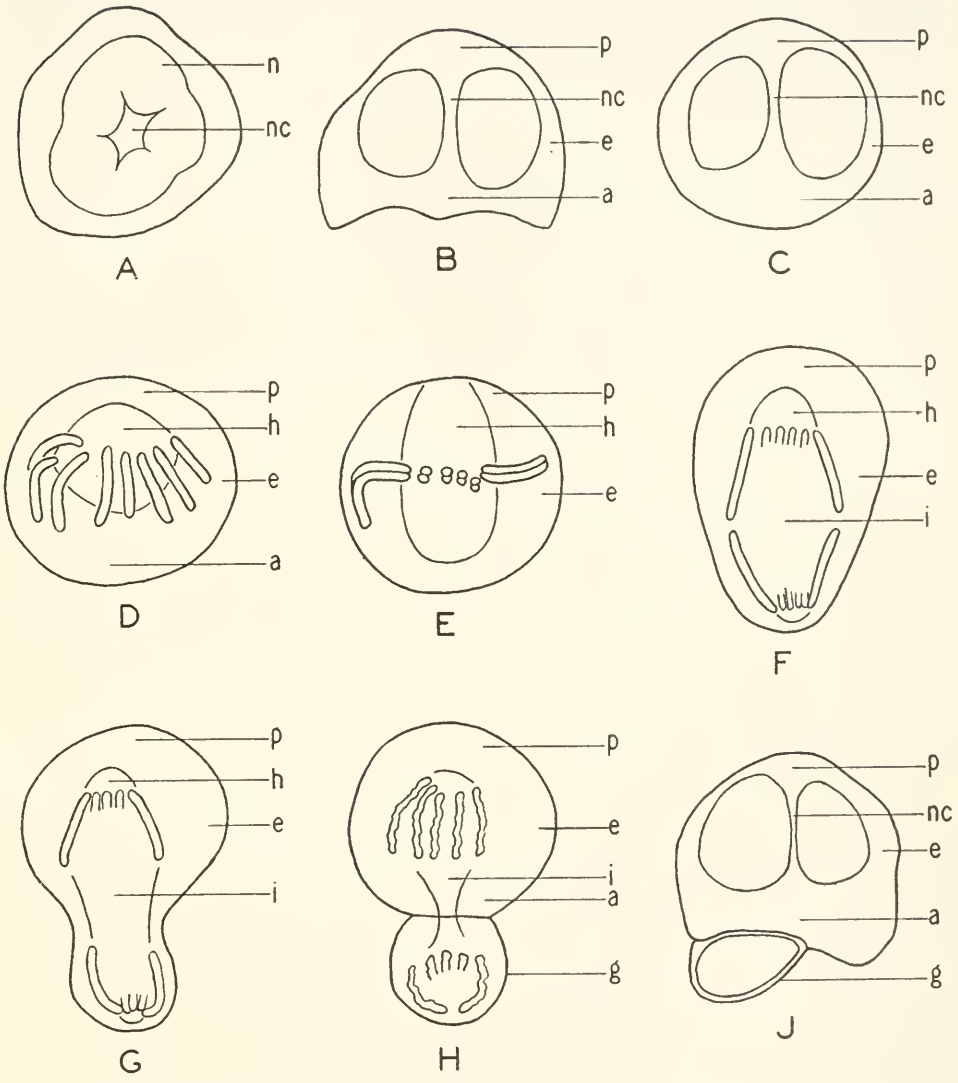

FIGURE 1. Representative stages in the mitotic cycle of the grasshopper neuroblast, reconstructed from camera lucida sketches of living cells growing in culture medium. *A* and *B*, polar and side views, respectively, of interphase or prophase cells; *C*, late prophase a few minutes before breakdown of the nuclear membrane, the cell having acquired a spherical form; *D*, prometaphase; *E*, metaphase; *F*, middle anaphase; *G*, late anaphase; *H*, early telophase; *J*, late telophase, the cell having lost its spherical form. *a*, polar cytoplasm; *e*, equatorial cytoplasm; *g*, ganglion cell; *h*, half spindle; *i*, interzonal region; *n*, nucleus; *nc*, nuclear core cytoplasm; *p*, polar cytoplasm.

viscosity values and the relative speeds of brownian movement are designated as follows:

Very high: No visible movement of mitochondria.

High: Movements very limited, discernible only with prolonged observation.

Medium: Movements readily observable, but not rapid.

Low: Movements quite free and rapid, but individual mitochondria can be followed with the eye.

Very low: Movements so rapid that individual mitochondria cannot be followed with the eye.

Even if the ability to make purely objective distinctions between adjacent members of this series be open to question, values separated by one class, viz., very high and medium, high and low, or medium and very low, can be distinguished readily and would give results essentially similar to those obtained.

Mitotic stages

The stages for which data were obtained and their distinguishing characteristics in the living, untreated state, when observed through 12.5 × compensating oculars and a 2 mm. oil immersion objective, are:

Interphase: Nuclear threads appear in optical section like small, rounded, scattered granules in an otherwise homogeneous nuclear background. Period of growth.

Very early prophase: Nuclear granules smaller in size. Threads have begun to appear in the previously homogeneous background.

Early prophase: Nucleus filled with extremely fine chromatin threads. No nuclear granules.

Middle prophase: Chromatin threads of appreciable thickness. Ends when about seven chromosomes in cross-section can be counted in one-fourth the nuclear circumference.

Late prophase: Chromosomes well-formed. Toward the end of this period cell assumes spherical shape (Fig. 1*B*, 1*C*). Ends with the simultaneous disappearance of the nuclear membrane and appearance of the spindle.

Prometaphase (Fig. 1*D*): Chromosomes straighten and move into the equatorial plane. Spindle develops.

Metaphase (Fig. 1*E*): Chromosomes in equatorial plane.

Early anaphase: Begins with the initial separation of the chromatids and ends when the distal ends of the chromosomes leave the cell equator.

Middle anaphase (Fig. 1*F*): This stage is terminated as the cleavage furrow penetrates to the interzonal region.

Late anaphase (Fig. 1*G*): Ends as the chromosomes lose their sharp outlines and the cleavage furrow appears to be complete.

Early telophase (Fig. 1*H*): This stage lasts until the nucleoli become visible.

Middle telophase: The nucleoli increase in size while retaining their spherical shape, the nuclear membrane re-forms, the interzonal spindle remnant disappears, and the cell re-assumes the hemispherical form.

Late telophase (Fig. 1*J*): Begins as the outlines of the nucleoli become irregular. Ends as linear arrangement of chromatin granules is lost.

The relative duration of these stages is given in an earlier paper (Carlson, 1941).

Regions of cell

Data have been obtained for six different regions of the cell, which are labeled in representative stages of the mitotic cycle in Figure 1.

Polar cytoplasm: This includes the region about the spindle pole of the daughter neuroblast when a spindle is present. At other stages it is the region within which the spindle pole of the daughter neuroblast will develop preparatory to the next division.

Apolar cytoplasm: The cytoplasm situated near the place of formation of the preceding cleavage furrow. It is opposite the polar region.

Equatorial cytoplasm: This is the portion of the cell that is situated at or near the cell equator in metaphase and anaphase and midway between the polar and apolar regions during the remainder of the mitotic cycle.

Nuclear core cytoplasm: The cytoplasmic core that passes through the nucleus (see p. 110).

Half spindle: This includes the developing spindle at prometaphase, the fully formed spindle at metaphase, and at anaphase the portion of the spindle lying between the pole and the plane in which the kinetochores of the adjacent chromosome group are situated.

Interzonal region: This is the part of the cell lying inside the ring of interzonal fibers and situated between the two separating groups of daughter chromosomes. It makes its appearance as the chromatids separate at early anaphase and persists as a spindle remnant into middle telophase.

Nucleus: No information that seemed reliable was secured for this part of the cell. Tiny nuclear granules comparable in size to the mitochondria showed very limited movement at various stages of the mitotic cycle. This might indicate a persistently high viscosity or it might mean merely that these granules are attached to the chromatin threads, which form a semi-rigid framework within the nucleus, and would therefore not be expected to exhibit much brownian movement. Bělař (1929a), however, has reported a rapid brownian movement of tiny granules of unknown nature situated in the ground substance of the nucleus of the grasshopper spermatocyte during prophase and telophase, indicating, according to him, that this substance is quite fluid.

Observations

The curves shown in Figure 2 were constructed in the following way. A linear series of arbitrary numerical values was assigned to the five selected viscosity classes described previously. Four observations were made for each region of the cell on different days and with different preparations. These were averaged and plotted. Because the determination of each of these was to a certain extent subjective, in that it depended on the judgment of the observer, and because the vis-

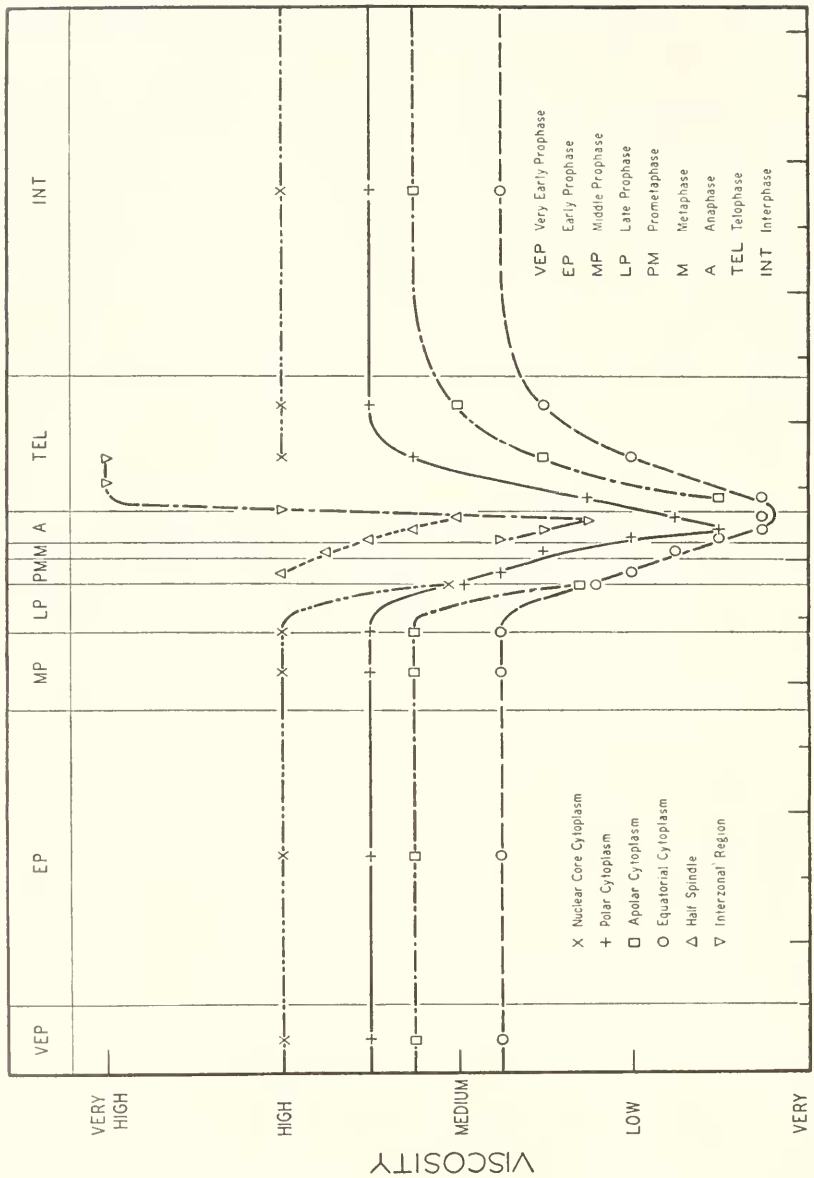
VISCOSITY
VERY HIGH
HIGH
MEDIUM
LOW
VERY LOW
TIME IN HOURS
VEP
EP
MP
LP
PM
M
A
TEL
INT
VEP Very Early Prophase
EP Early Prophase
MP Middle Prophase
LP Late Prophase
PM Prometaphase
M Metaphase
A Anaphase
TEL Telophase
INT Interphase
X Nuclear Core Cytoplasm
+ Polar Cytoplasm
□ Apolar Cytoplasm
○ Equatorial Cytoplasm
△ Half Spindle
▽ Interzonal Region

FIGURE 2

cosity varied slightly from preparation to preparation and from cell to cell, certain of these curves showed irregularities. In order to discover whether these were the result of experimental error or of actual viscosity shifts, new cultures were prepared, checked, and any necessary corrections made. It is believed that the graph in its present form represents quite accurately the cycle of viscosity changes in the different regions of the cell during mitosis. It should be emphasized, however, that the five arbitrarily-chosen viscosity values represented by ordinates are not necessarily a linear series of absolute values, as their graphical treatment implies.

During the greater portions of interphase and prophase the viscosity of the cytosome maintains a relatively high and constant value. In late prophase, a few minutes before the disappearance of the nuclear membrane, a rapid fall in viscosity is initiated. This change begins as the cell changes in shape from hemispherical (Fig. 1*B*) to spherical (Fig. 1*C*). The increase in the rapidity of mitochondrial movement is distinguishable first at the juncture of the equatorial and apolar regions as this part of the cell begins to draw in coincidentally with the start of the rounding-up process. From this region the lowered viscosity spreads into the apolar region, which thickens as the rounding-up proceeds, and subsequently into the equatorial and polar regions. Obviously, some of this is due to the actual movement of the cytoplasm, which must of necessity shift in position as the cell shape changes, but this can hardly be responsible for more than a small fraction of the viscosity change that takes place at this time. Since this alteration in shape occupies only about 5–10 minutes, this sequence of changes does not appear in the graph. The viscosity continues to fall through prometaphase and metaphase to reach a minimum in anaphase. The progress of the cell through telophase is accompanied by a steady rise in viscosity until the interphase-prophase level is attained. During telophase the hemispherical form of the cell is re-assumed (Fig. 1*J*).

The half spindle appears to show a slight fall in viscosity from prometaphase to early telophase, by which time it is too small for further study.

As the chromosome halves begin to separate at early anaphase, the interzonal region between the groups often contains one or more mitochondria showing a moderate amount of brownian movement. During middle and late anaphase large numbers of mitochondria move into the interzonal region, until they are as concentrated as outside, except for a small region inside each of the separating chromosome groups. Their movement is quite rapid, but less so than that of the mitochondria outside in the equatorial region. As the cleavage furrow presses in against the interzonal fibers, the transverse diameter of the interzonal region decreases and the motion of the mitochondria quickly slows down and then stops entirely. The fact that these stationary bodies are lined up in rows parallel with the long axis of the spindle makes it appear as if they had been caught among interzonal fibers of higher viscosity as these were pressed inward by the deepening cleavage furrow. By late telophase these fibers are no longer visible.

The high viscosity level of the nuclear core cytoplasm immediately after the formation of the nucleus and core may be due to the fact that this is the region occupied earlier by the spindle remnant of the preceding division.

Discussion

The brownian movement method

Of the three most frequently used methods of making determinations of protoplasmic viscosity changes, viz., centrifuge, microdissection, and brownian movement, the last has the advantage of producing no physical disorientation of the cell contents. It can be depended on to give reliable results, however, only if certain factors are taken into consideration:

1) The protoplasm surrounding the granules in brownian movement must be homogeneous, if the viscosity of the protoplasm as a whole, exclusive of the granules, is being determined. If regions of different viscosity are present, observations of the rate of brownian movement of a particle will indicate only the viscosity of the material immediately surrounding it. The polar, equatorial, apolar, and nuclear core regions of the neuroblast cytosome are visibly homogeneous except for the mitochondria, and the fact that these bodies appear to migrate about and pass one another in the course of their shorter zig-zag movements demonstrates that they are not enclosed in a substance of one viscosity that is in turn surrounded by material of another viscosity. The spindle, however, is probably an exception to this (see p. 118).

2) Alterations in the size of the mitochondria from one stage of mitosis to another would cause an apparent shift in the viscosity of the surrounding medium, even though no actual change took place. Such changes could conceivably result either from shrinking and swelling as a consequence of osmotic shifts or from division and growth. The latter can be ruled out in the case of the neuroblast because very few mitochondria would need to divide in each cell generation to make up for the loss to the daughter ganglion cell, which receives very little cytoplasm and very few mitochondria. The resulting effect on brownian movement would be insufficient to alter the general viscosity picture. With regard to osmotic changes, Lewis and Lewis (1915) report that immersing cells in hypotonic solution causes swelling of the mitochondria. Bělař (1929a), on the other hand, states that mitochondria are particularly insensitive to the usual swelling effects of hypotonic solution. Unfortunately, the neuroblast mitochondria are so small and move so rapidly at certain stages that exact comparisons of size by actual measurements are impossible. Careful visual comparisons of the mitochondria of adjoining cells in different mitotic stages, however, have failed to reveal any significant size differences. As far as the present study is concerned, swelling of the mitochondria of late prophase, prometaphase, and metaphase cells by intake of water (p. 119) would tend to reduce rather than augment the observed viscosity shift in these stages; therefore, the actual change would be even greater than that shown in Figure 2.

3) If the granules under observation are so crowded as to interfere with each other's movements, the viscosity values obtained at the lower levels will be too high; for frequent collisions will retard their movements. The mitochondria of the neuroblast are definitely crowded, and this could easily result in appreciable errors in absolute viscosity values based on the migration of a given body a certain distance in a certain time, according to the method described by Pekarak (1930). Much of this error is doubtless avoided in the present study, however, because viscosity determinations were based on the rapidity with which the particles as a group dance about rather than on the total distance they migrate in a given interval of time.

Results of related studies

Of the several studies made on viscosity changes of the whole cytoplasm during mitosis, the centrifuge determinations of Heilbrunn (1917, 1921) for the marine invertebrate egg (Arbacia, Cumingia, and Nereis) and of Kostoff (1930) for meiotic cells of Nicotiana and the brownian movement determinations of Kato (1933) for the meiotic cells and staminate hairs of Tradescantia have given results that are similar to those I have obtained for the polar, apolar, and equatorial cytoplasm of the grasshopper neuroblast. All these studies show that the viscosity is relatively high during interphase, when interphase is referred to at all, high during all or most of prophase, falling during metaphase, lowest at anaphase, and rising at telophase.[2] The similarities in these results are all the more striking in view of the great diversity of the cell types used. The marine invertebrate egg contains relatively large quantities of ergastic matter, such as oil globules and yolk and pigment granules, and develops during mitosis an astral system and spindle that involve a large portion of the protoplast. The pollen mother cell, though consisting of a more or less homogeneous cytosome with relatively small spindle and no astral system, nevertheless is atypical mitotically because of the extended prophase and the two successive meiotic divisions without an intervening interphase. The staminate hairs of the plant contain large vacuoles with only a peripheral film and a few central strands of cytoplasm. The grasshopper neuroblast contains a fairly homogeneous cytosome with no visible ergastic matter or vacuoles but an abundance of mitochondria. The spindle is moderate in size and no asters are visible. This suggests strongly that viscosity change is a fundamental factor in mitosis, and is largely independent of individual pecularities of the type of cell studied.

Zimmermann (1923), who studied the viscosity of the alga, Sphacelaria, by means of the centrifuge and brownian movement, and Seifriz (1920), whose studies of the marine invertebrate egg are based on microdissection, place the viscosity fall and minimum somewhat in advance of this mitotically. According to Zimmermann the viscosity is lowest at metaphase, while Seifriz finds it lowest during late prophase and metaphase. Both describe the viscosity as rising at anaphase and high at telophase.

In contrast with these findings are the results obtained by Chambers (1917, 1919) from studies of Arbacia and Echinarachnius eggs with the microdissection needle. He reported that the viscosity of the greater part of the cell is low during prophase, rising during metaphase, high during anaphase and early telophase, and falling in late telophase. He related the viscosity to the state of development of the amphiaster: low when no amphiaster is present, rising as the amphiaster forms, highest when it has reached its maximum development, and falling as the amphiaster disappears at the end of mitosis. He does, however, describe a liquefaction of the equatorial region previous to anaphase and persisting through cleavage.

Fry and Parkes (1934) duplicated as closely as possible the centrifuge studies of Heilbrunn on Arbacia, Cumingia, and Nereis eggs. The results they obtained for viscosity in relation to time after fertilization were identical with those reported earlier by Heilbrunn; in fact, in their paper they used Heilbrunn's curves for Arbacia and Nereis eggs. They interpreted this data, however, as supporting

[2] Heilbrunn associates the pre-cleavage fall and the post-cleavage rise in viscosity of the Arbacia egg with spindle development and disappearance, respectively.

Chambers' conclusions, claiming that Heilbrunn had misidentified certain of the mitotic stages. This would place viscosity changes of the marine invertebrate egg in entire disagreement with those of the grasshopper neuroblast, in which there can be no question of the correct identification of the different mitotic stages.

Kostoff's description of viscosity changes in somatic cells of Nicotiana (1930) is not in accord with any of these results. Using the centrifuge technique, he reported two cycles of viscosity change for each mitotic cycle: high viscosity at prophase and anaphase, and low viscosity at metaphase and interphase.

Observations on the viscosity of the spindle are complicated by the probable presence in the half spindle of two materials, spindle fibers and interfibrillar substance. If there are two such materials present, the mitochondria or other cytoplasmic granules that make their way into the spindle by brownian movement would be expected to occupy the region of lower viscosity, i.e., the interfibrillar region. Under such conditions their speed of movement would be determined by two factors: the viscosity of the interfibrillar substance and the amount of space available for movement between the fibers, especially if this were very limited.

Bělař (1929b) found that most spindles in cells of the stamen hairs of Tradescantia contained a few tiny granules in quite rapid brownian movement. This motion of the granules, which appeared first in the polar caps (clear regions that adjoin opposite sides of the nucleus at late prophase and in which the spindle later develops), was evident in the fully-formed spindle and continued undiminished up to the time of formation of the cell plate, when the granules disappeared from view. This contrasted with the situation he found in the spindles of animal cells, namely, nematode eggs, lepidopteran and grasshopper spermatocytes, and Actinophrys, in which granules of a comparable size in the spindle were always relatively quiet (see Bělař, 1929a). He interpreted this difference to indicate that in the Tradescantia cell there was a larger amount of the less viscous interfibrillar substance than in the animal cells he studied. I have not been able to confirm Bělař's observation that the granules show greater freedom of movement in the direction of the long axis of the spindle than at right angles to it, but this may mean only that the interfibrillar substance is more abundant and the fibers farther apart in my material than in his.

Ris (1943) reports unrestricted brownian movement of the cytoplasmic granules that make their way into the interzonal region during anaphase in embryonic cells of Tamalia. Chambers (1924) states that the spindle of the dividing sand-dollar egg has become "distinctly fluid" by the time the chromosomes have reached the poles. He doubtless refers to what I have termed the interzonal region. These observations agree well with the conditions in the grasshopper neuroblast.

Possible causal factors in viscosity change

With regard to the factors responsible for viscosity changes of the cytoplasm, two possibilities seem deserving of consideration: alterations in the water content and in the pentose nucleic (ribonucleic) acid content of the cytoplasm.

It has already been pointed out (p. 115) that the first detectable viscosity drop coincides with the initiation of the "rounding-up" of the neuroblast in late prophase and that the viscosity is rising as the cell flattens on one side to assume the hemispherical form during telophase. Since it can be demonstrated that immersion of

these cells in hypotonic culture medium causes a fall in viscosity owing to intake of water and that hypertonic medium causes a rise in viscosity through loss of water, it seems not unlikely that the late prophase viscosity fall and "rounding-up" of the cell might result from the intake of water, while the telophase viscosity rise and the accompanying flattening of the cell at one side might be due to loss of water. Unfortunately, the irregularities in the hemispherical-shaped cell make it impossible to determine whether there is any actual change in cell volume during the alteration in shape. Chambers (1919) relates the change in form of the Arbacia egg from spherical to hemispherical following the first cleavage division to a viscosity shift, but he associates it with a lowering of the viscosity.

Water exchange seems inadequate, however, as a complete explanation of the observed viscosity changes for two reasons. First, it seems doubtful that the intake of water would occur in sufficient amounts to account entirely for the observed viscosity fall. Second, the viscosity continues to rise for some time after the cell has returned to its hemispherical shape, when the water loss might be supposed to have been completed. Another factor, therefore, would appear to be involved, either in place of or in addition to water intake and loss.

In support of the possibility that changes in the pentosenucleic acid content of the cytoplasm may be at least partly responsible for the observed viscosity changes is the fact that pentosenucleic acid, which is known to be present in large quantities in the cytoplasm of rapidly growing tissues (Brachet, 1933; Caspersson and Schultz, 1940) and which has a strikingly high viscosity ($\eta = 62.4$, according to Cohen and Stanley, 1942), undergoes a change in amount per cell during mitosis (Brachet, 1940; Caspersson, 1940; Painter, 1943). Both Brachet, from studies of the eggs and early developmental stages of different animals, and Painter, using Rheo meiotic cells, reached the conclusion that cytoplasmic pentosenucleic acid is abundant in mitotically active cells at early prophase, less abundant or entirely absent from late prophase through anaphase, and increasing in amount following division. They believe that the late prophase decrease is due to transformation of pentosenucleic acid into the desoxypentosenucleic acid of the developing chromosomes and that the increase following division is due to the loss of desoxypentosenucleic acid from the chromosomes and its transformation into pentosenucleic acid. These changes in the nucleic acid content of the cytoplasm during mitosis bear a very close resemblance to my viscosity curve for the neuroblast cytoplasm. If there is any discrepancy, it would seem to be the exact time during late prophase that the nucleic acid leaves the cytoplasm in detectable amounts, and neither the studies of Brachet nor Painter furnish information on this point. The statement of White (1942), however, that "the nucleic acid of the chromosomes undergoes a sudden increase at prometaphase, when the nuclear membrane breaks down and substances from the cytoplasm have free access to the chromosomes" suggests that the fall in cytoplasmic nucleic acids may coincide closely with the viscosity fall of the cytoplasm. The viscosity rise, which extends through all of telophase, could be accounted for, up to the time of nuclear membrane formation, by a return of the pentosenucleic acids to the cytoplasm during the retrogressive chromosome changes, and, after re-constitution of the nuclear membrane, by the synthesis of new pentosenucleic acid.

Summary

Observations on the rapidity of brownian movement of the mitochondria in different regions of the grasshopper neuroblast during the entire mitotic cycle indicate that:

1) The viscosity of all parts of the cytosome is relatively high during interphase and prophase, begins to fall in late prophase, reaches a minimum at anaphase, and rises gradually to its original high level during telophase.

2) The viscosity of the portion of the spindle between the pole and the plane in which the proximal ends of the chromosomes are situated appears to fall slightly from a high prometaphase level through anaphase.

3) The viscosity of the portion of the spindle situated between the separating daughter chromosome groups shows, during anaphase, a slight drop from a medium value, and this is followed at the beginning of telophase by an abrupt rise to a very high level, which is maintained through early and middle telophase.

Alterations in water content and nucleic acid content of the cytoplasm are suggested as possible explanations of viscosity changes during mitosis.

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